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In-situ aldehyde-modification of self-assembled acyl hydrazide hydrogels and dynamic component selection from complex aldehyde mixtures

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Self-assembled hydrogels based on the industrially-relevant 1,3:2,4-dibenzylidene sorbitol framework functionalised with reactive acyl hydrazides (DBS-CONHNH$_2$) peripheral groups react with aldehydes without disrupting the nanoscale gel network, adapting gel performance, and dynamically selecting specific aldehyde components from complex mixtures.

Low-molecular-weight gelators (LMWGs) that self-assemble into supramolecular gels via non-covalent interactions are fascinating soft materials.[1] They have a wide range of applications, for example in personal care and drug delivery as a result of their rheological properties and ability to encapsulate active agents.[2] Supramolecular gels benefit from simple synthesis, high tunability and dynamic/responsive properties; significant advantages over widely-used polymer-based gels.[3] Solvents and other active agents can diffuse through the liquid-like phase of gels, enabling environmental remediation[4] or catalysis[5] as a result of the high surface area of the nanoscale fibres, which can interact effectively with pollutants/reagents.

In recent times, there has been considerable interest in multi-component materials, in which different molecular-scale components cooperate to form the gel.[6] Such systems can have intriguing selectivity and dynamics – for example, component selection, in which a gelator preferentially selects components from complex mixtures, incorporating them into its gel network.[7] In particular, self-assembled hydrogels, that form in water, have potential biological/environmental compatibility and applications.[2] Many LMWG hydrogels include pH-responsive carboxylic acids that self-assemble as pH is lowered and solubility decreases.[8] Acyl hydrazides have similar solubility profiles to carboxylic acids and can therefore be interesting, pH-stable, replacements. In key research, Lehn, Herrmann and co-workers used acyl-hydrazide-functionalised gels to react reversibly with aldehydes for fragrance immobilisation and release – a dynamic covalent approach relying on the formation of a reversible covalent bond.[9] Other researchers have also explored acylhydrazide gels.[10] van Esch and co-workers used the reaction between acylhydrazides and aldehydes to form an acylhydrazone hydrogel; in this work the acylhydrazide acts as a pre-gelator and does not form gels in its own right.[11] In a landmark paper, the controlled diffusion of different aldehydes gave spatially-defined acylhydrazone gels with different chemical compositions at different locations.[12] There has also been general interest in developing supramolecular gels which are underpinned by dynamic covalent chemistry.[13]

We have recently been working on commercially-relevant, gels based on 1,3;2,4-dibenzylidenesorbitol (DBS),[14] reporting the first true DBS-based hydrogels.[15] Acylhydrazide-functionalised DBS-CONHNH$_2$ is an excellent hydrogelator, stable across the pH range (pH 3-12), and formed via a simple heat-cool cycle,[16] with the acyl hydrazide functional group introduces useful functionality. For example, DBS-CONHNH$_2$ gels can extract pollutant dyes,[16] control pharmaceutical release,[17] and reduce precious metals *in situ* to give conducting gold nanoparticles[18] or catalytically-active Pd nanoparticles.[19] In this new study (Fig. 1), we wanted to explore the reactivity of the acylhydrazide group, and understand how these hydrogels behave when challenged with different aldehydes.

We synthesised DBS-CONHNH$_2$ using the simple two-step procedure reported previously[16] and formed gels by dissolving it in water on heating (8.42 mM, 4 mg/0.5 mL) and then cooling. The gels were exposed to different aldehydes (Fig. 1), dissolved in water (16.84 mM, 2 eq., 0.5 mL), pipetted carefully on top of the gel and allowed to diffuse into it. To allow full diffusion of the aldehyde into the gel, the supernatant was left for 48 h on top of the gel and then removed. All hydrogels remained stable, indicating that self-assembly was not disrupted. MS analysis indicated peaks for DBS-CONHNH$_2$ and also the acylhydrazone derivatives (see ESI). Thus, attachment of aldehyde to the gelator, forming mono-/di-substituted acylhydrazones occurs.

^{1}H NMR spectroscopy was used to quantify the amount of aldehyde reacted (Fig. 2, top). This was achieved by vacuum

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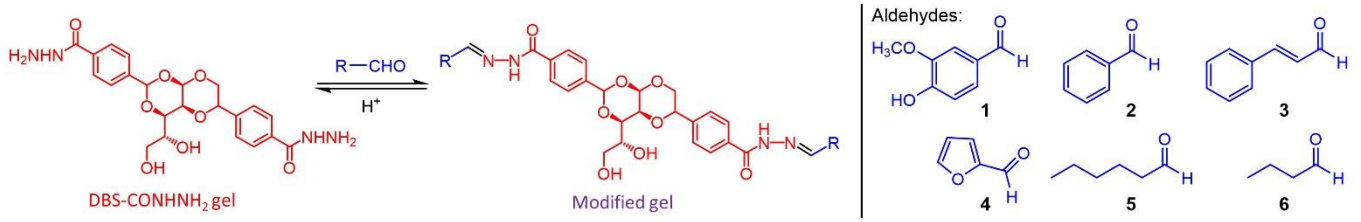

Figure 1. DBS-CONHNH$_2$ reacts reversibly with aldehydes in the hydrogel-phase, to form acylhydrazone linkages on the periphery of self-assembled gel nanofibres, giving rise to modified hydrogel performance. Aldehydes: **1** = vanillin, **2** = benzaldehyde, **3** = cinnamaldehyde, **4** = furfural, **5** = hexanal, **6** = butanal.

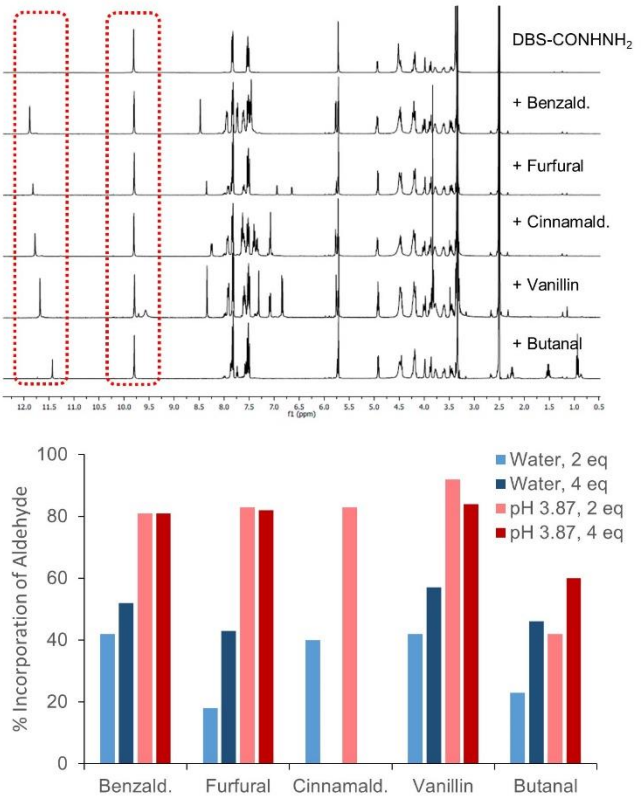

Fig. 2. (Top) ^{1}H NMR spectra of DBS-CONHNH$_2$ and aldehyde-modified xerogels, dissolved in d_6-DMSO. (Bottom) Conversion of DBS-CONHNH$_2$ into aldehyde-modified form by diffusion in water (blue) and pH 3.87 citrate buffer (red).

drying the aldehyde-loaded gel to yield a xerogel, and dissolving it in d_6- DMSO (Fig. S1). Integration of the downfield-shifted acylhydrazone peaks and comparison with the unreacted acylhydrazide determined that ca. 40% of the LMWG reacted with aromatic aldehydes, falling to ca. 20% with butanal and furfural (Fig. 2, bottom, light blue bars). In an attempt to maximise uptake, the aldehyde concentration was then doubled (33.68 mM, 4 eq.). This increased loadings by ca. 10-20% (Fig. 2, bottom, dark blue bars), although cinnamaldehyde was too insoluble in water to load at this concentration. In all other experiments, the aldehydes have sufficient solubility. Furthermore, these aldehydes do not possess electron withdrawing groups and are thus expected to be stable with respect to hydrate formation. Clearly this approach would not be suitable for insoluble or unstable aldehydes.

The experiment was then performed at lower pH, as it is known pH affects acylhydrazone formation.[20] Pleasingly, DBS-CONHNH$_2$ gels still formed using a simple heat-cool cycle even in citrate buffer (pH 3.87). ^{1}H NMR xerogel analysis indicated loadings >80% for all aromatic aldehydes (Fig. 2, bottom, light red bars). Increasing aldehyde concentration did not enhance loading, except for butanal, which increased from 42% to 60% (Fig. 2, bottom, dark red bars). The aliphatic aldehyde is less well incorporated than aromatic ones, suggesting alkyl hydrazone formation is less favoured, as might be expected because of lower conjugation in the product. Lowering pH further using a phthalate buffer (pH 3.0) was unsuccessful, leading to by-products – citrate buffer was therefore optimal.

We were concerned that the drying step in our analytical workflow might impact apparent uptake. We thus applied a ^{1}H NMR method to confirm aldehyde uptake onto the nanofibres in the solvated gel phase. The DBS-CONHNH$_2$ gel was formed in citrate buffer (pH 3.87 in D_2O) in an NMR tube, and a supernatant aldehyde solution placed on top. The system was left for 14 days, to ensure aldehyde diffusion through the gel. After this time, the amount of free aldehyde in the supernatant and the gel was determined by ^{1}H NMR with integration against citrate buffer as internal standard. We then assume that any unaccounted-for aldehyde is probably attached to the gel fibres (it may have precipitated out in the gel network but we observe no visual evidence of this and consider it unlikely). The self-assembled acylhydrazone-form of the aldehyde is not visible in the gel-phase ^{1}H NMR as it is immobile on the NMR timescale. The results of this study were in broad agreement with those above, indicating that the aldehyde becomes attached to gel nanofibres, with more effective attachment of aromatic aldehydes (Table S1). This therefore gave us confidence in our more rapid analytical approach using sample drying (Fig. S1).

The aldehyde-modified hydrogels were then characterised using simple reproducible tube-inversion methodology to yield T_{gel} values. A DBS-CONHNH$_2$ hydrogel (8.42 mM) prepared in citric acid buffer (pH 3.87), had a T_{gel} of 74°C. This compares to the gel in H_2O, which has a T_{gel} value of 70°C. After aldehyde uptake, a significant increase in T_{gel} was observed, demonstrating that the gel changes on aldehyde addition. Indeed, diffusion of all aromatic aldehydes into the gel led to modified hydrogels with T_{gel} values > 100 °C. However, on addition of butanal, the T_{gel} value only increased to 80 °C. We suggest that acylhydrazone formation increases inter-gelator non-covalent interactions like $\pi-\pi$ stacking and solvophobicity, particularly for the aromatic aldehydes, hence increasing thermal stability. This will be less significant for butanal, which is taken-up less effectively by the gel, and also lacks an aromatic ring to reinforce assembly.

Rheology was then investigated using a parallel plate geometry (Fig. S2). G' increased after diffusion of the aromatic aldehydes into the gel from 1050 Pa for DBS-CONHNH$_2$ alone in citrate buffer, to 1880 Pa for the vanillin-modified gel, 2570 Pa for benzaldehyde, 2830 for furfural, and 6600 Pa for the cinnamaldehyde-modified gel. This suggests a significant increase in stiffness caused by nanofibre modification, in agreement with enhanced non-covalent interactions between acylhydrazones. Butanal addition induced no significant rheological change (G' = 1040 Pa) in the gels.

Comparative SEM studies of dried samples of DBS-CONHNH$_2$ in the absence and presence of vanillin (as a typical aromatic aldehyde) indicated that both samples had nanoscale fibrillar morphologies, but after aldehyde modification, fibre width increased (from ca. 50 nm to ca. 500 nm) and fibre length decreased (Fig. S3). However, drying effects are hard to avoid and are significant in these samples.[21] Indeed, in support of this view, although the aldehyde-modified gels remain stable as gels while solvated, if they are dried down, the resulting acyl hydrazone xerogels are then unable to reform gels. We reason that the solubility of the modified gelator in water is lower, and once dried, the energy barrier to gelator solubilisation and subsequent nanofibre assembly can no longer be overcome.

Having characterised the interaction of DBS-CONHNH$_2$ with each aldehyde individually, we then explored how the gel would behave if challenged with a mixture. We reasoned that the dynamic nature of the acyl hydrazide/aldehyde reaction may allow thermodynamics to drive preferential aldehyde uptake, giving *component selection*. We thus pipetted aldehyde pair mixtures on top of the hydrogel in citrate buffer at pH 3.87 – each aldehyde was present at two molar equivalents with respect to gelator, as such, total aldehyde was present in excess (4 eq.), forcing DBS-CONHNH$_2$ to 'choose' between aldehdyes, a situation where component selection may operate.

Table 1 lists the percentage of each aldehyde attached to the gel nanofibres (determined by ^{1}H NMR of the dried xerogel in d_6-DMSO). With uptakes between 55% and 84% vanillin was always most favoured. Benzaldehyde, cinnamaldehyde and furfural were all taken up to similar extents, slightly less effectively than vanillin (30-85%). Hexanal was taken up significantly less well (32-44%) while butanal was the least competitive aldehyde (15-27%) – correlating with the fact that aliphatic aldehydes had the lowest uptake on their own, gave the modified gel with the lowest T_{gel} value, and had little rheological impact. We were unable to directly compete butanal against hexanal, because the NMR peaks of the resulting acylhydrazones overlap. Overall, the results were in-line with the conversions observed on treating DBS-CONHNH$_2$ with individual aldehydes: i.e., vanillin > benzaldehyde/ cinnamaldehyde/furfural > hexanal > butanal – suggesting the effects that control the loading experiments read through into component selection preferences.

Table 1. Competition between pairs of aldehydes (total concentration 16.84 mM) for interaction with DBS-CONHNH$_2$ (8.42 mM) in citrate buffer (pH 3.87).

		Competitor Aldehyde					
		1	**2**	**3**	**4**	**5**	**6**
Measured Aldehyde	Vanillin (**1**)	-	55	62	70	68	84
	Benzaldehyde (**2**)	45	-	50	50	61	85
	Cinnamaldehyde (**3**)	30	50	-	51	61	75
	Furfural (**4**)	38	50	49	-	56	73
	Hexanal (**5**)	32	39	39	44	-	n/a
	Butanal (**6**)	16	15	25	27	n/a	-

The same experiments were also performed at pH 7 (Table S2). Butanal had lower uptake under these conditions and cinnamaldehyde higher. The reaction is more fully reversible at pH 7,[20] and *component selection* effects may be greater than at pH 3.8, where although total uptake is higher, there is a degree of kinetic control. Enhanced cinnamaldehyde uptake of would agree with the rheology described above that indicated this aldehyde gave the stiffest gels.

We then investigated mixtures of three aldehydes in citrate buffer (pH, 3.87, Table S3). Once again, vanillin was favoured (49%-70% uptake) – well above the statistical average of 33%. Benzaldehyde, cinnammaldehyde and fufural were taken up to similar intermediate extents (when the three are directly competed against each other, there was a slight preference for benzaldehyde, 38%). Hexanal and butanal were taken up the least effectively (12%-29%) - always below the statistical average of 33%. Where hexanal and butanal were both present in the experiment, the peaks for individual hydrazones could not be distinguished, and the sum of products was determined.

To demonstrate the dynamic nature of the process, we then determined if the most favoured aldehyde, vanillin could displace other aldehydes from gel fibres once they were already attached. DBS-CONHNH$_2$ hydrogels were functionalized with the aldehyde of choice (2. eq., 16.84 mM, pH = 3.87). The supernatant was removed and replaced by a vanillin solution (2. eq., 16.84 mM, pH = 3.87). Vanillin replaced 19% of furfural, 25% of benzaldehyde, 31% of cinnamaldehyde, 75% of hexanal and 100% of butanal. This suggests these gels are dynamic, and one aldehyde can displace another even if the acylhydrazone linkage has already formed. However, it is worth noting that forming the aromatic acylhydrazones first, prior to adding vanillin, leads to systems that are compositionally different to those formed when both aldehydes are added simultaneously (i.e. there is less vanillin – c.f. Table 1). This suggests that the

system does not completely equilibrate, and the history helps determine the composition. Once again, however, the aromatic aldehydes strongly outcompete aliphatic systems.

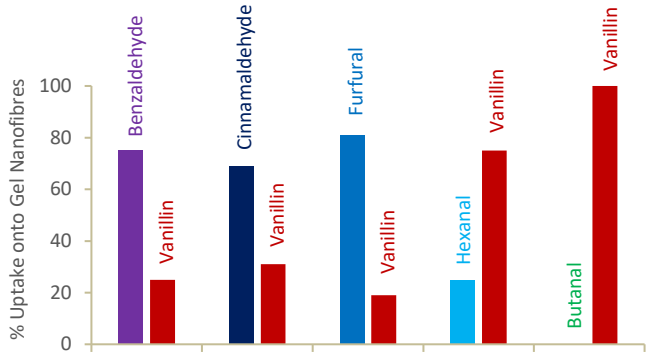

Fig. 3. Dynamic displacement of aldehydes from gel fibres by vanillin. Firstly the gel was loaded with one of aldehydes **2**-**6**, then vanillin was loaded on top of the modified gel and left to stand. The gel was dried and analysed, and the relative amounts of each aldehyde attached to the gel nanofibres was determined.

We then attempted to replace vanillin with the other aldehydes using the same approach (Table S4), with ca. 20% of vanillin being replaced by benzaldehyde, furfural or cinnamaldehyde, and ca. 10-15% of vanillin by hexanal or butanal.

In summary, we report the facile and dynamic modification of gels based on DBS-$CONHNH_2$ with aldehydes, giving acylhydrazones that retain their capacity to support a gel, that has modified thermal and rheological properties. Thermodynamic preferences for aldehyde loading read through into the way DBS-$CONHNH_2$ behaves when challenged with aldehyde mixtures, with preferential uptake being observed. Dynamic aldehyde uptake is demonstrated, with favoured aldehydes being better able to displace less favoured ones than *vice versa* after gel uptake. Further work should focus on the detailed kinetic analysis to understand the evolution of these materials over time. This constitutes a simple way of modifying DBS-$CONHNH_2$-based gels, and suggests controlled this dynamic hydrogel may have application in the controlled release of water soluble/stable aldehyde-based active ingredients.[9b] The use of functionalised aldehydes would be a simple and effective way of introducing further functionality to these hydrogels

Conflicts of interest

There are no conflicts to declare.

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Graphical Abstract

Acyl hydrazide functionalised hydrogels can react with aldehydes yielding modified gels with adapted performance, and can dynamically select specific aldehyde components from mixtures.